KU LEUVEN

Citation	Markou Giorgos*, Vandamme Dries, Muylaert Koenraad (2014) **Using natural zeolite for ammonia sorption from wastewater and as nitrogen releaser for the cultivation of Arthrospira platensis** Bioresource technology, vol 155 (issue), 373-378.
Archived version	Author manuscript: the content is identical to the content of the published paper, but without the final typesetting by the publisher
Published version	insert link to the published version of your paper: http://dx.doi.org/10.1016/j.biortech.2013.12.122
Journal homepage	http://www.sciencedirect.com/science/article/pii/S0960852413019627
Author contact	your email markoug@aua.gr; dries.vandamme@kuleuven-kulak.be Klik hier als u tekst wilt invoeren.
IR	url in Lirias https://lirias.kuleuven.be/handle/123456789/432934

(article begins on next page)

Accepted Manuscript

Using natural zeolite for ammonia sorption from wastewater and as nitrogen releaser for the cultivation of *Arthrospira platensis*

Giorgos Markou, Dries Vandamme, Koenraad Muylaert

PII: S0960-8524(13)01962-7
DOI: http://dx.doi.org/10.1016/j.biortech.2013.12.122
Reference: BITE 12845

To appear in: *Bioresource Technology*

Received Date: 24 October 2013
Revised Date: 24 December 2013
Accepted Date: 28 December 2013

Please cite this article as: Markou, G., Vandamme, D., Muylaert, K., Using natural zeolite for ammonia sorption from wastewater and as nitrogen releaser for the cultivation of *Arthrospira platensis*, *Bioresource Technology* (2014), doi: http://dx.doi.org/10.1016/j.biortech.2013.12.122

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Using natural zeolite for ammonia sorption from wastewater and as nitrogen releaser for the cultivation of *Arthrospira platensis*

Giorgos Markou[1*], Dries Vandamme[2] and Koenraad Muylaert[2]

[1] Department of Natural Resources Management and Agricultural Engineering, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

[2] Laboratory Aquatic Biology, KU Leuven Kulak, , E. Sabbelaan 53, 8500 Kortrijk, Belgium

[*] E-mail: markoug@aua.gr (G. Markou)

Abstract

Herein a new approach for the application of wastewater nutrients for the cultivation of cyanobacteria or microalgae is described. Natural zeolite was used as medium for the sorption of ammonia from wastewater and subsequently as nitrogen releaser in cultures of *Arthrospira. platensis*. The main scope of the present approach was to isolate ammonia from the wastewater and to transfer it into the culture medium excluding thus the suspended solids, the dissolved colored compounds or any other possible contaminant of the wastewater. The results demonstrate that the indirect use of ammonia derived from wastewater using zeolite as sorption and releasing medium for the cultivation of *A. platensis* is

ACCEPTED MANUSCRIPT

promising. This is the first time that a medium was used for indirect application of wastewater nutrient for the production of cyanobacterial or microalgal biomass.

Keywords: Ammonia inhibition; A .platensis; biomass; cyanobacteria; microalgae; wastewater; zeolite

1. Introduction

Microalgae and cyanobacteria are a massive source for various compounds which could be used in the food, pharmaceutical, industrial and bio-energy sector. Microalgae and cyanobacteria are photosynthetic microorganisms that utilize solar energy and along with several elements (N, P, C etc.) synthesize valuable organic molecules, such as proteins, lipids, pigments etc. (Gouveia and Oliveira, 2009; Mata et al., 2010).

Nitrogen is the second most abundant element in microalgal and cyanobacterial biomass (Grobbelaar, 2004) and represents a major cost for biomass production (Borowitzka and Moheimani, 2013). A strategy to decrease the application of nitrogen is to use wastewater streams (Park et al., 2010; Park et al., 2011). However, various types of wastewater and especially from the agro-industrial sector contain relative high concentration of ammonia, which is known to be inhibitory to microalgae and cyanobacteria. Ammonia above a specific concentration can have inhibitory or even detrimental results to the cells (Azov and Goldman, 1982). Anaerobic digestion is a widely used technology for wastewater treatment and the digested effluents are suggested as potential source of nutrients

ACCEPTED MANUSCRIPT

for microalgal and cyanobacterial biomass production (Singh et al., 2011; Wang et al., 2010). During the anaerobic digestion a fraction of the organic matter is microbiologically converted to inorganic forms, including ammonia which is the degradation product of the nitrogenous organic compounds. Anaerobically digested wastewaters contain frequently ammonia above 2 g l^{-1} (Yenigün and Demirel, 2013).

Moreover, beside the high ammonia content wastewaters from the agro-industrial sector contain considerable amounts of suspended solids and dissolved colored compounds, which reduce the light penetration into the culture resulting in a decrease of growth rates (Depraetere et al., 2013). Furthermore, the production of microalgal or cyanobacterial biomass contaminated with solids of the wastewater raises questions about the ability of using this biomass for several industrial applications. Additionally, wastewaters might contain several biological contaminants which could negatively affect the microalgal or cyanobacterial cultures (Wang et al., 2013). Therefore in some cases an indirect way of applying wastewater nutrients may be desirable.

Zeolite is used traditionally in agriculture as soil conditioner and as nitrogen retaining medium for nitrogen fertilizing improvements (Polat et al., 2004). Zeolite has a high cation-exchange capability and is therefore capable of adsorbing ammonia (in particular the protonated form (NH_4^+)) and thus it has been suggested to be used for wastewater treatment, lowering their ammonia load (Wang and Peng, 2010). Some researchers have also suggested applying zeolite for ammonia sorption in order to mitigate its inhibitory effects on the anaerobic microorganisms during the anaerobic digestion (Milán et al., 2001). More recently, zeolites are suggested to

ACCEPTED MANUSCRIPT

be used as supplement to the cultivation medium for growth stimulation of silicon-demanding marine micro-algae, such as diatoms (Fachini and Vasconcelos, 2006). However, to the best knowledge of the authors, no previous study exists, in which a sorption medium like zeolite was investigated to be used for wastewater nutrient sorption and subsequently as nutrient releaser in cultures of microalgae or cyanobacteria, taking possibly advantage of the isolation of nutrients and their transferring from the wastewater into the culture medium. Aim of the present study was to examine and demonstrate the capability of zeolite as an indirect way of applying wastewater nutrients for microalgal and cyanobacterial biomass production.

2.1 Microorganism

The cyanobacterium *Arthrospira platensis* SAG 21.99 used in the study was obtained from SAG (Sammlung von Algenkulturen der Universität Göttingen). The inoculum for the experiments was prepared by the cultivation of *A. platensis* in Zarrouk medium under 60 μmol photons m^{-2} s^{-1} light illumination. As inoculum, cells in the exponential growth stage were used. The Zarrouk medium had the following composition: 16.8 g l^{-1} $NaHCO_3$, 2.5 g l^{-1} $NaNO_3$, 0.5 g l^{-1} K_2HPO_4, 1.0 g l^{-1} K_2SO_4, 1.0 g l^{-1} NaCl, 0.04 g l^{-1} $CaCl_2$, 0.08 g l^{-1} Na_2EDTA, 0.2 g l^{-1} $MgSO_4 \cdot 7H_2O$, 0.01 g l^{-1} $FeSO_4 \cdot 7H_2O$ and 1.0 ml of trace elements: 2.86 g l^{-1} H_3BO_3, 0.02 g l^{-1} $(NH_4)6Mo_7O_{24}$, 1.8 g l^{-1} $MnCl_2 \cdot 4H_2O$, 0.08 g l^{-1} Cu_2SO_4 and 0.22 g $l^{-1}ZnSO_4 \cdot 7H_2O$.

2.2 Experimental set-up

ACCEPTED MANUSCRIPT

2.2.1 Enrichment of zeolite with ammonia

The enrichment of the natural zeolite with ammonia was made by the addition of 150 g of zeolite in one liter of either synthetic wastewater or real wastewater. The natural zeolite used had its origin from Serbia and was of the type clinoptilolite with purity of 92–94%, having the following chemical composition (as given from the supplier): SiO_2 64.88%, Al_2O_3 12.99%, CaO 3.26%, Fe_2O_3 2.00%, MgO 1.07%, Na_2O 0.95%, K_2O 0,89%, TiO_2 0,37%. Some of the physical properties of the zeolite are: bulk density: 1,42 g cm^{-3}, porosity: 45–50%, hardness: 3 – 3,5 Mohs, and its total cation exchange capacity: 1,85 – 1,92 meq g^{-1}. The zeolite used in the study had a particle size of 4-6mm.

The artificial wastewater had the following composition (modified from He et al. (2005)): NH_4Cl 3000 mg N l^{-1}; K_2HPO_4 100 mg P l^{-1} ; $CaCl_2$ $2H_2O$, 150 mg l^{-1}; KCl, 300 mg l^{-1}; NaCl, 300 mg l^{-1}; $MgSO_4$ $7H_2O$, 1630 mg l^{-1} and 1 ml l^{-1} trace metals (see Zarrouk receipt). Real wastewater (effluents from anaerobically digested poultry manure) had an ammonia concentration of 7430 ± 49 mg l^{-1} (n=3). Beside the very high ammonia concentration, the effluents were brown-dark colored with total dissolved and suspended solids content of 3-3.5 % (w/w).

For the ammonia enrichment process, the pH of the real and the synthetic wastewater was adjusted to 8. The amounts of zeolite (150 g l^{-1}) used for the enrichment was selected so that at the end of the enrichment process not all ammonia would be removed in order to ensure that the zeolite adsorbed ammonia at the maximum of its capacity.

ACCEPTED MANUSCRIPT

The enrichment of the zeolite was conducted in sealed 2 l conical flasks which were placed on an agitation plate and were agitated overnight at room temperature. The ammonia sorption (enrichment) onto zeolite was calculated as the difference between the initial ammonia concentration in the wastewaters and their ammonia concentration after 24 h. The zeolite prior to the experiments was rinsed several times with DI water to wash-out unwanted particles.

2.2.2 Ammonia desorption kinetics

In a series of experiments the ammonia desorption kinetics of the ammonia-enriched zeolite (AEZ) were investigated. AEZ was placed in modified Zarrouk medium (without $NaNO_3$) in concentrations of 10, 25, 50 and 100 g l^{-1}. Samples of 40 ml were placed in 50 ml plastic centrifuge tubes and agitated by an agitation plate. Desorption of ammonia from zeolite to the solution was calculated as follow:

$$\text{Desorption (\%)} = \frac{\text{AEZ ammonia} - \text{ammonia in solution}}{\text{AEZ ammonia}} * 100$$

where "AEZ ammonia" is the amount of ammonia bounded in the zeolite and "ammonia in solution" is the ammonia concentration measured in the modified Zarrouk medium at time t. The pH of the modified Zarrouk medium was not adjusted and was 8.6-8.7.

2.2.3 Cultivation of *A. platensis*

ACCEPTED MANUSCRIPT

After the ammonia enrichment the AEZ was washed 3 times with 500 ml DI water each time, in order to ensure that the ammonia for the cultivation of *A. platensis* derived from the zeolite and not from wastewaters residuals. Zeolite was added to the cultivation medium wet and its expression in dry weight was based on its water absorption capability which was measured to be 63.04% ± 0.47% (n=3; dried at 105 °C overnight). The wet AEZ was added to already inoculated with *A. platensis* cultures. Due to gravity zeolite settled to the bottom of the PBR.

A. platensis was cultivated in batch mode in the modified Zarrouk medium. The modification of the medium refers to the replacement of $NaNO_3$ with AEZ as the nitrogen source. Four concentration of zeolite was used, namely 10, 25, 50 and 100 g l^{-1}.

The experiments were carried out in 250 ml semi-closed photobioreactors (PBR). The working volume was set on 150 ml. The cultures were aerated, in order to be agitated, with filtered air provided by a membrane air pump. The cultivation was carried out in air-conditioned room and culture temperature was kept constant at 30 °C (± 2 °C). Light intensity was set at 100 μmol photons m^{-2} s^{-1} (measured in the middle of the PBR) and was provided continuously through a 57W fluorescence tube-lamp on the one side of the PBR. Runs were carried out in duplicates.

2.3 Analytical methods

Dry algal biomass was measured indirectly by spectrophotometry at 560 nm. Chlorophyll was determined spectrophotometrically after extraction in hot absolute methanol. Proteins were determined according to the Lowry method using bovine

ACCEPTED MANUSCRIPT

albumin as standard. Total lipids were determined according to the sulfo-phospho-vanillin reaction method, after lipid extraction with the Bligh and Dyer method using triolein as standard. Carbohydrates were determined by the phenol-sulfuric acid method using D-glucose as standard (for references about the analytical methods see Markou et al. (2012)). All biomass composition analyses were performed after the washing of the samples for several times with DI water. Ammonia was measured with the phenate method according to Solorzano (1969). In the present study, with the term ammonia the total dissolved ammonia is meant, i.e. the sum of the protonated NH_4^+ and free NH_3 ammonia species concentration, which their ratio is determined by the pH values of the solution (pKa≈9.25). All spectrophotometric determinations were carried out on a Dr. Lange, Cadas 30 (Germany) spectrophotometer and analyses were carried out at least in triplicates.

2.4 Ammonia recovery calculations

The amounts of ammonia (mgN) that was taken up by *A. platensis* was assumed to be equal to the biomass nitrogen content, which was calculated in the basis of the biomass protein content divided by the factor 5.95 (González López et al., 2010). Ammonia recovery was calculated according to the following equation:

$$\text{Ammonia recovery} = \frac{\text{Ammonia taken up}}{\text{Ammonia added}}$$

Explicitly, ammonia recovery was the ratio of ammonia taken-up by *A. platensis* and the amount of ammonia added to the cultures as AEZ. The ammonia recovery was expressed as mgN taken-up per gN of added ammonia.

Specific ammonia uptake was calculated according to the following equation:

ACCEPTED MANUSCRIPT

$$\text{Specific ammonia uptake} = \frac{\text{Ammonia taken up}}{\text{Biomass} * \text{AEZ}}$$

Explicitly, specific ammonia uptake was the ratio of the taken-up ammonia per g of biomass and g of added AEZ. In the calculations the losses of ammonia by stripping were not included.

3. Results and discussion

3.1 Ammonia sorption onto zeolite

Zeolite is suggested to be used for ammonia and/or phosphorus sorption to treat wastewaters (Wu et al., 2006). In the present study it was aimed to investigate the simultaneous sorption of nitrogen and phosphorus from anaerobically digested effluents. However, it was observed that maximum phosphorus sorption onto the zeolite used in this study was very low (103 ± 6 μg g^{-1}) and therefore no further investigation were performed for phosphorus applications through zeolite sorption.

Ammonia enrichment process showed that the ammonia sorption onto zeolite was 6.89 ± 0.64 and 8.89 ± 1.84 mgN g^{-1} of zeolite for synthetic and real wastewater, respectively. This difference probably was due to the overall different chemical composition of the two wastewaters, and mainly due to their difference on the organic load, resulting thus to different ammonia adsorption degrees (Huang et al., 2010; Wu et al., 2006). The ammonia amounts adsorbed by the zeolite in this study are inside the range of reported values in the literature, which range from about 3 to 26 mgN g^{-1}. This wide range of values reported are due to the fact that ammonia sorption degree is not only affected from the chemical composition of the

ACCEPTED MANUSCRIPT

ammonia solution, but it is also affected by the type of zeolite (natural or synthetic) and their origin, having diverse adsorption capacities (Wang and Peng, 2010).

3.2 Desorption of ammonia

Desorption kinetics of ammonia were performed in order to investigate the rate of ammonia release from zeolite into the cultivation medium. One of the main hypotheses of the present study was that zeolite could act as a slow nitrogen releaser taking possible advantages. However, ammonia was relative fast released to the cultivation medium. In the first 15 minutes almost 50% of bounded ammonia was released to the medium, while after 120 minutes about 80% of the bounded ammonia was released to the medium reaching also the highest desorption capability. The zeolite concentration had no effect on the ammonia desorption kinetics. The same desorption kinetics as the AEZ from real wastewater were obtained also with AEZ from synthetic wastewater (data not shown).

It is known that natural zeolites (clinoptilolites) have a selectivity for the adsorption of cations, with the following sequence: $K^+ > NH_4^+ > Na^+ > Ca^{2+}$, Mg^{2+}(Kithome et al., 1998). Zarrouk medium contains adequate amounts of potassium, a fact that explains the observation that ammonia was fast released to the cultivation medium. That means that zeolite, at least in solutions such as Zarrouk medium which contain adequate amounts of potassium or any other exchangeable cation, cannot be considered as a slow nitrogen releaser.

3.3 Cultivation of *A. platensis* using untreated zeolite

ACCEPTED MANUSCRIPT

To examine the effect of the zeolite *per se* on the cyanobacterial cells, *A. platensis* was cultivated in Zarrouk medium (with $NaNO_3$ as nitrogen source) supplied with untreated zeolite in concentration of 10, 25, 50 and 100 g l^{-1}. As shown in **Figure 1**, the presence of zeolite inside the cultures affected the growth capability of *A. platensis*. However, the only significant decrease in growth was observed when zeolite concentration was in its highest level (100 g l^{-1}), while the differences of lower zeolite concentrations with the control cultures (without zeolite) were low and gradually lowered as the concentration of added zeolite decreased (**Figure 1**). The growth decrease perhaps was a result of the cation-exchanging activity of the zeolite, i.e. the adsorption or liberation of trace metals from and on the cultivation medium (Fachini and Vasconcelos, 2006) in concentration which might inhibited the growth of *A. platensis*. However, the results show that zeolite can be added to the Zarrouk medium up to 50 g l^{-1} without to cause *per se* a significant decrease of *A. platensis* growth.

3.3 Cultivation of *A. platensis* using ammonia-enriched zeolite

To investigate the capability of *A. platensis* to be cultivated in medium supplemented with AEZ, four AEZ concentrations were used, namely 10, 25, 50 and 100 g l^{-1}. The different amounts of AEZ added to the modified Zarrouk medium (without $NaNO_3$) did not affect the pH of the medium, probably due to the fact that Zarrouk medium is strongly buffered because of the high concentration of soda bicarbonate and the subsequently formation of bicarbonate-carbonate buffer. After the addition of AEZ the cultivation media had an initial value of 8.6-8.7 for all cultures. However, during

ACCEPTED MANUSCRIPT

the cultivation the pH values increased with time, due to photosynthesis and reached 9.5 and above.

As shown in **Figure 2**, after the addition of AEZ, *A. platensis* was negatively affected, a phenomenon which however was not observed in cultures with the addition of untreated zeolite (data not shown). It was observed in a macroscopic level that immediately (couple of minutes) after the addition of the AEZ the biomass began to be attached and adsorbed onto the surface of the zeolite, resulting thus to a decrease in the concentration of the suspended biomass (**Figure 2**).The attached to the zeolite biomass was re-suspended during the cultivation period, however a portion of the biomass was still attached in the surface of zeolite. The decrease in the suspended biomass of *A. platensis* was gradually and increased as the AEZ concentration increased. Since, this behavior of *A. platensis* was not observed in cultures with the presence of untreated zeolite, it is assumed that the biomass adsorption onto zeolite was due to the bounded ammonia onto zeolite and not due to the zeolite *per se*. It is supposed that in the cultures with AEZ some form of chemotaxis was occurred, by which *A. platensis* was directed and attached to the surface of AEZ in order to take up the bounded ammonia. It is displayed that some cyanobacteria and microalgae are significantly attracted by and motivated toward to nitrogenous compounds and in particular NH_4^+ (Sjoblad and Frederikse, 1981; Willey and Waterbury, 1989). However, some chemical interaction between the exchangeable bounded ammonia and cell walls of *A. platensis* could be also occurred resulting to the sorption of biomass onto AEZ. This point needs more investigation. 120 min after the inoculation the growth of *A. platensis* was revived, except in runs

ACCEPTED MANUSCRIPT

with 100 g l^{-1} AEZ which were absolutely inhibited (**Figure 3**), indicating that in these cultures ammonia acted toxic with detrimental results to the cells.

Concerning the relative fast ammonia release from the zeolite onto the cultivation medium (see section 3.2), growth of *A. platensis* was gradually inhibited by the presence of ammonia. Due to this, the lag phase of *A. platensis* was gradually increased as zeolite concentration increased, except in run with AEZ 10 g l^{-1}. In this AEZ concentration no lag phase was observed, probably due to that the released ammonia concentration was low and did not inhibit significantly the growth of *A. platensis*. The concentration of the released ammonia in the cultures with 10 g l^{-1} AEZ was about 54 and 69 mgN l^{-1}, from synthetic and real wastewater, respectively (calculated based on the amount of bounded ammonia in AEZ and 78% desorption). At these relative low ammonia concentration the decrease in the photosynthetic capacity of *A. platensis* is expected to be less than 30% (Belkin and Boussiba, 1991). The growth rates of *A. platensis* for the first 24 hours of cultivation with 10 g l^{-1} AEZ from synthetic and real wastewater were equal or even higher (0.053 h^{-1} and 0.057 h^{-1}, respectively) than the growth rate of the control run without zeolite (0.053 h^{-1}) and with untreated zeolite (0.046 h^{-1}). However, the highest growth rates observed (for 72 hours of cultivation) were in cultures with AEZ of 25 g l^{-1}.

The specific cultivation conditions (light intensity, temperature etc.) of the present study could support biomass density over 1100 mg l^{-1} after 72 hours of cultivation as was obtained in control runs (with $NaNO_3$ as nitrogen source and without the presence of zeolite). The lower biomass density obtained using zeolite indicates that beside the ammonia inhibition a limitation in the growth capability of

ACCEPTED MANUSCRIPT

A. platensis was occurred and it seems that the main limitation factor that affected *A. platensis* growth was the nitrogen supply. Based on the biomass composition analyses (**Table 1**), *A. platensis* was nitrogen starved; in all runs, except control runs, the biomass was rich in carbohydrates, a fact that indicates that the cultures were indeed nitrogen starved (van Rijn and Shilo, 1986). The nitrogen starvation is assumed to be occurred due to ammonia losses (stripping) from the medium during the cultivation. Ammonia losses in *A. platensis* cultures due to high pH values of the cultivation medium (over 9.5) can be as high as 80% and are higher as ammonia concentration in the medium increases (data submitted for publication elsewhere). It is assumed that the cells of *A. platensis* were negatively affected by the released from the zeolite ammonia resulting to a lower growth and lower ammonia uptake from the medium. At that point the released ammonia was lost by stripping owing the high pH (8.6-8.7) and the aeration of the cultures (Pouliot et al., 1989) and its concentration was gradually reduced. The reduced ammonia concentration resulted to the reducing of the ammonia inhibitory effect on cells and subsequently growth of *A. platensis* was revived. However, the ammonia concentration left in the medium was not enough to support biomass production causing a nitrogen limited growth of *A. platensis*.

Between runs with AEZ from synthetic and real wastewater, the growth was in general more smoothly and maximum biomass production and growth rates were higher when zeolite was enriched from real wastewater (**Figure 4; Table 2**). This might be attributed to the presence of the organic matter in the wastewater, which

ACCEPTED MANUSCRIPT

during the process of zeolite enrichment caused a decrease of the toxic activity of some compounds of the zeolite (Lin et al., 2012).

The presence of suspended solids or dissolved colored compounds in the wastewater is proven that reduces the light penetration inside the culture affecting negatively the growth of *A. platensis* (Depraetere et al., 2013). Thus, a potential benefit of the indirect application of wastewater nutrients and their separately transferring from the wastewater into the culture medium is the overcoming of this growth limitation. Also, a potential benefit of the proposed method is that unwanted contaminants (suspended particles, various toxic compounds or biological contaminants) are not transferred from wastewater into the cultures. This perhaps results to the production of biomass for human consumption or any other application which presupposes quality biomass. However, this point needs more research.

3.4 Ammonia recovery

The maximum ammonia recovery obtained in the present study was in cultures with AEZ of 10 g l^{-1} derived from synthetic wastewater and amounted 362 $mgN_{taken\text{-}up}$ gN_{added}^{-1} (**Figure 5**). However, as shown in this figure ammonia and specific ammonia recovery in all cases had almost the same trend and decreased as AEZ concentration increased. As was mentioned above, it is apparent that the increase of AEZ concentration caused a gradual ammonia loss and consequently the recovery of ammonia from AEZ decreased as AEZ concentration increased. A probably strategy, which has to be proved in future work to overcome this issue, is the gradual

ACCEPTED MANUSCRIPT

addition of AEZ to the cultures, on the one hand to reduce the ammonia inhibition and on the other hand to reduce ammonia losses.

A significant issue for choosing and using media for indirect application of wastewater nutrients, beside their cost, is their sorption and desorption capability. High sorption and desorption capability would mean that less amounts of the medium will be used reducing perhaps the overall costs (initial and process costs). Also a significant characteristic of the medium should be the capability to be regenerated (Cyrus and Reddy, 2011; Kithome et al., 1998) so that it can be reused for several cultivation cycles.

There are several wastewater treatment methods (De-Bashan and Bashan, 2004; Renou et al., 2008; Saracco and Genon, 1994) by which the removed nutrient could be used to fulfill the needs for microalgal or cyanobacterial biomass production through an indirect way as is suggest in the present work. For example struvite (magnesium ammonium phosphate) which is a product of the precipitation of ammonia and/or phosphorus could be used as fertilizer (De-Bashan and Bashan, 2004; Renou et al., 2008). Also there are plenty of substrates that are studied for nutrient removal from wastewater (Johansson Westholm, 2006; Wang and Peng, 2010), which however have to be evaluated for their capability to be used as media for indirect application of nutrients for microalgal or cyanobacterial biomass production.

The results of the present study suggest that *A. platensis* cultivation using an indirect way of applying wastewater nutrients is demonstrated successfully. This is the first report of such an approach and further studies are needed for the

ACCEPTED MANUSCRIPT

optimization of such a system. For further work it is suggested to screen and find adsorption media capable to adsorb and desorb adequate amounts of nutrients, which could support the production of satisfactory amounts of microalgal or cyanobacterial biomass.

4. Conclusions

This study demonstrated the "proof of principle" for the indirect use of ammonia derived from wastewater through its adsorption onto zeolite for the cultivation of *A. platensis*, eliminating the contamination of the cultures from unwanted matter of the wastewater. The maximum recovery of ammonia reached about 35%, however it seems that ammonia stripping was occurred and significant amounts of ammonia were lost, resulting to the production of nitrogen starved biomass. This is the first time that a medium was used for indirect applying of wastewater nutrient for the production of microalgal or cyanobacterial biomass. Further study is needed for the optimization of such a process.

Acknowledgements

G.M. would like to thank Professor Dimitris Georgakakis for his support. D.V is a Postdoctoral Researcher funded by the Research Foundation – Flanders (FWO).

References

ACCEPTED MANUSCRIPT

1. Azov, Y., Goldman, J.C., 1982. Free ammonia inhibition of algal photosynthesis in intensive cultures. Appl. Environ. Microbiol., 43, 735-739.
2. Belkin, S., Boussiba, S., 1991. High internal pH conveys ammonia resistance in *Spirulina platensis*. Bioresour. Technol., 38, 167-169.
3. Borowitzka, M., Moheimani, N., 2013. Sustainable biofuels from algae. Mitigation and Adaptation Strategies for Global Change, 18, 13-25.
4. Cyrus, J.S., Reddy, G.B., 2011. Sorption and desorption of ammonium by zeolite: Batch and column studies. Journal of Environmental Science and Health, Part A, 46, 408-414.
5. De-Bashan, L.E., Bashan, Y., 2004. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). Water Res., 38, 4222-4246.
6. Depraetere, O., Foubert, I., Muylaert, K., 2013. Decolorisation of piggery wastewater to stimulate the production of Arthrospira platensis. Bioresour. Technol., 148, 366-372.
7. Fachini, A., Vasconcelos, M.T.S., 2006. Effects of zeolites on cultures of marine micro-algae. Environmental Science and Pollution Research, 13, 414-417.
8. González López, C.V., García, M.d.C.C., Fernández, F.G.A., Bustos, C.S., Chisti, Y., Sevilla, J.M.F., 2010. Protein measurements of microalgal and cyanobacterial biomass. Bioresour. Technol., 101, 7587-7591.
9. Gouveia, L., Oliveira, A., 2009. Microalgae as a raw material for biofuels production. J. Ind. Microbiol. Biotechnol., 36, 269-274.

ACCEPTED MANUSCRIPT

10. Grobbelaar, J.U., 2004. Algal niutrition. Mineral nutrition. in: *Handbook of microalgal culture: Biotechnology and applied phycology*, (Ed.) A. Richmond, Blackwell Publishing Ltd. Oxford, pp. 97-115.
11. He, Z., Minteer, S.D., Angenent, L.T., 2005. Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environ. Sci. Technol., 39, 5262-5267.
12. Huang, H., Xiao, X., Yan, B., Yang, L., 2010. Ammonium removal from aqueous solutions by using natural Chinese (Chende) zeolite as adsorbent. J. Hazard. Mater., 175, 247-252.
13. Johansson Westholm, L., 2006. Substrates for phosphorus removal—Potential benefits for on-site wastewater treatment? Water Res., 40, 23-36.
14. Kithome, M., Paul, J., Lavkulich, L., Bomke, A., 1998. Kinetics of ammonium adsorption and desorption by the natural zeolite clinoptilolite. SSSAJ, 62, 622-629.
15. Lin, D., Ji, J., Long, Z., Yang, K., Wu, F., 2012. The influence of dissolved and surface-bound humic acid on the toxicity of TiO2 nanoparticles to Chlorella sp. Water Res., 46, 4477-4487.
16. Markou, G., Chatzipavlidis, I., Georgakakis, D., 2012. Effects of phosphorus concentration and light intensity on the biomass composition of *Arthrospira* (*Spirulina*) *platensis*. World J. Microbiol. Biotechnol., 28, 2661-2670.

ACCEPTED MANUSCRIPT

17. Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: A review. Renew. Sust. Energ. Rev., 14, 217-232.

18. Milán, Z., Sánchez, E., Weiland, P., Borja, R., Martın, A., Ilangovan, K., 2001. Influence of different natural zeolite concentrations on the anaerobic digestion of piggery waste. Bioresour. Technol., 80, 37-43.

19. Park, J., Jin, H.-F., Lim, B.-R., Park, K.-Y., Lee, K., 2010. Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp. Bioresour. Technol., 101, 8649-8657.

20. Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011. Wastewater treatment high rate algal ponds for biofuel production. Bioresour. Technol., 102, 35-42.

21. Polat, E., Karaca, M., Demir, H., Naci-Onus, A., 2004. Use of natural zeolite (clinoptilolite) in agriculture. J. Fruit Ornam, 12, 183-189.

22. Pouliot, Y., Buelna, G., Racine, C., de la Noüe, J., 1989. Culture of cyanobacteria for tertiary wastewater treatment and biomass production. Biological Wastes, 29, 81-91.

23. Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P., 2008. Landfill leachate treatment: Review and opportunity. J. Hazard. Mater., 150, 468-493.

24. Saracco, G., Genon, G., 1994. High temperature ammonia stripping and recovery from process liquid wastes. J. Hazard. Mater., 37, 191-206.

ACCEPTED MANUSCRIPT

25. Singh, M., Reynolds, D.L., Das, K.C., 2011. Microalgal system for treatment of effluent from poultry litter anaerobic digestion. Bioresour. Technol., 102, 10841-10848.

26. Sjoblad, R., Frederikse, P.H., 1981. Chemotactic responses of *Chlamydomonas reinhardtii*. Mol. Cell. Biol., 1, 1057-1060.

27. Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr, 14, 799-801.

28. van Rijn, J., Shilo, M., 1986. Nitrogen limitation in natural populations of cyanobacteria (Spirulina and Oscillatoria spp.) and its effect on macromolecular synthesis. Appl. Environ. Microbiol., 52, 340-344.

29. Wang, H., Zhang, W., Chen, L., Wang, J., Liu, T., 2013. The contamination and control of biological pollutants in mass cultivation of microalgae. Bioresour. Technol., 128, 745-750.

30. Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J., Ruan, R.R., 2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresour. Technol., 101, 2623-2628.

31. Wang, S., Peng, Y., 2010. Natural zeolites as effective adsorbents in water and wastewater treatment. Chem. Eng. J., 156, 11-24.

32. Willey, J., Waterbury, J., 1989. Chemotaxis toward nitrogenous compounds by swimming strains of marine Synechococcus spp. Appl. Environ. Microbiol., 55, 1888-1894.

ACCEPTED MANUSCRIPT

33. Wu, D., Zhang, B., Li, C., Zhang, Z., Kong, H., 2006. Simultaneous removal of ammonium and phosphate by zeolite synthesized from fly ash as influenced by salt treatment. J. Colloid Interface Sci., 304, 300-306.

34. Yenigün, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochem., 48, 901-911.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table and Figure captions

Table 1. Biomass composition of *A. platensis* cultivated with ammonia-enriched zeolite (n=6; ±SD).

Table 2. Growth rates (μ, h^{-1}) of *A. platensis* cultivated under various concentration of untreated and ammonia-enriched zeolite. Growth rates were calculated for cultivation period of 72 hours ((± SD%)

Figure 1. Biomass production of *A. platensis* cultivated in Zarrouk medium with the presence of various concentrations of untreated zeolite. In the figure are also included control runs of *A. platensis* cultivated in Zarrouk medium without zeolite and in Zarrouk medium without zeolite and nitrogen.

Figure 2. Biomass production of *A. platensis* cultivated in modified Zarrouk medium with the presence of various concentrations of ammonia-enriched zeolite (enriched from synthetic wastewater).

Figure 3. Biomass production with natural zeolite enriched with (a) ammonia form synthetic wastewater and (b) ammonia from real wastewater.

ACCEPTED MANUSCRIPT

Figure 4. Maximum biomass produced by *A. platensis* cultivated with various concentrations of ammonia-enriched zeolite.

Figure 5. Ammonia (dashed line) and specific ammonia recovery (solid line) from *A. platensis* cultivated in modified Zarrouk medium with the addition of ammonia-enriched zeolite.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Run	Carbohydrates ($\%_{dw}$)	Proteins ($\%_{dw}$)	Lipids ($\%_{dw}$)	Chlorophylls ($\%_{dw}$)
$R_{Control}$	18.77 ± 3.65	42.17 ± 3.38	2.02 ± 0.13	0.95 ± 0.08
Synthetic wastewater				
R_{10}	55.29 ± 7.87	26.59 ± 4.07	6.73 ± 0.48	0.46 ± 0.05
R_{25}	58.35 ± 2.07	28.69 ± 2.50	6.94 ± 0.84	0.53 ± 0.05
R_{50}	62.80 ± 4.61	19.19 ± 0.84	6.30 ± 0.24	0.48 ± 0.05
R_{100}	-	-	-	-
Real wastewater				
R_{10}	65.31 ± 6.15	18.84 ± 4.88	5.82 ± 0.37	0.57 ± 0.04
R_{25}	61.56 ± 4.45	26.62 ± 2.81	5.77 ± 0.52	0.65 ± 0.03
R_{50}	57.03 ± 2.35	22.08 ± 1.35	5.00 ± 0.60	0.66 ± 0.06
R_{100}	-	-	-	-

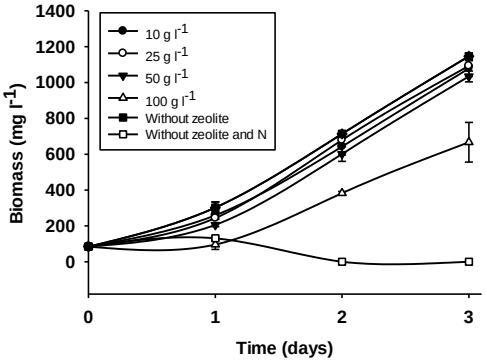
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Zeolite concentration ($g\ l^{-1}$)	Untreated zeolite	AEZ synthetic	AEZ real	Control
0				0.036 (± 1.64%)
10	0.035 (± 4.06%)	0.025 (± 19.15%)	0.029 (± 2.39%)	
25	0.035 (± 0.38%)	0.029 (± 11.76%)	0,030 (± 2.00%)	
50	0.035 (± 2.93%)	0.025 (± 3.23%)	0.020 (± 2.01%)	
100	0.028 (± 16.60%)	-	-	

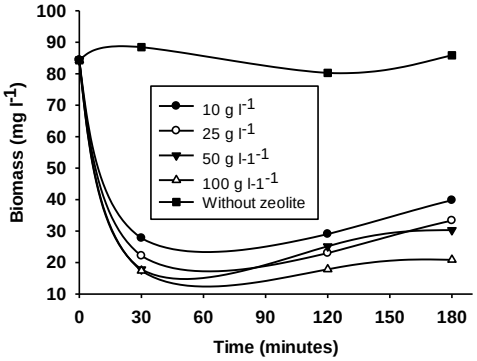
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

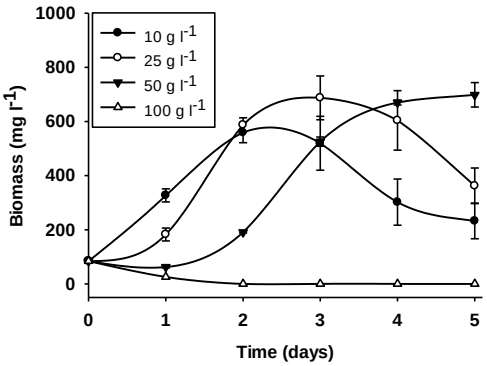
ACCEPTED MANUSCRIPT



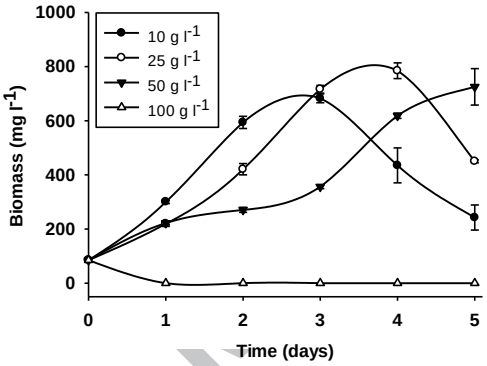
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

a)

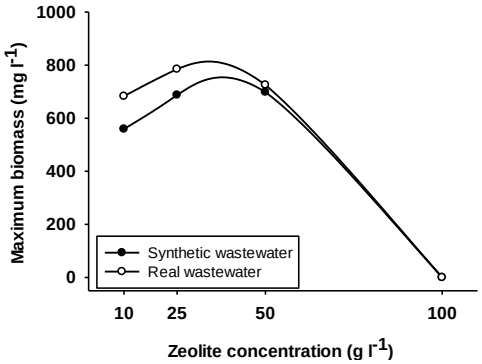


b)



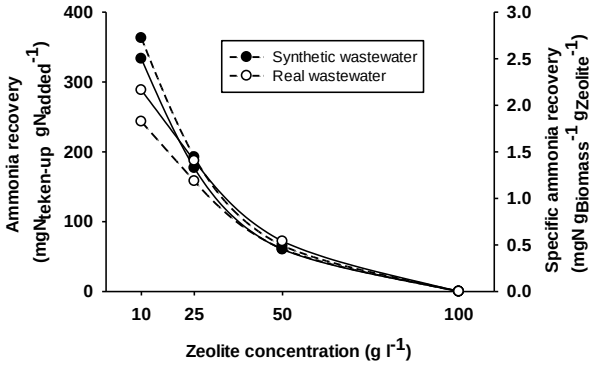
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

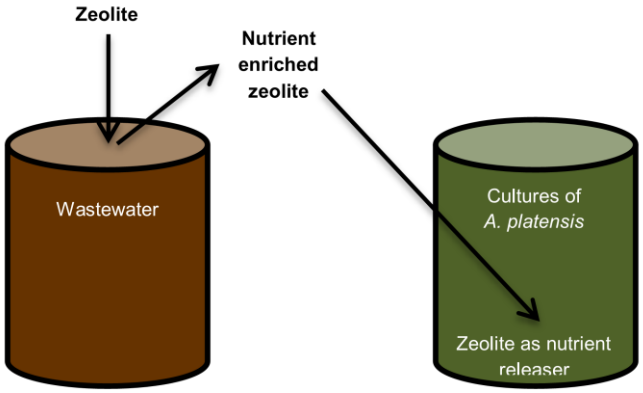
Highlights

- A new approach is described for microalgal biomass production using wastewater
- Zeolite was used to adsorb ammonia from ammonia-rich wastewater
- The ammonia enriched zeolite (AEZ) was transferred to cultures of *A. platensis*
- AEZ released ammonia to the cultures and acted as the sole nitrogen source

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Graphical abstract



ACCEPTED MANUSCRIPT